

DIVISION S-8—NOTES

ACID FUMIGATION OF SOILS TO REMOVE CARBONATES PRIOR TO TOTAL ORGANIC CARBON OR CARBON-13 ISOTOPIC ANALYSIS

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Abstract

The use of ^{13}C natural abundance ($\delta^{13}\text{C}$) to follow C input to soil has gained widespread acceptance. However, inorganic C present in the soil as carbonates will interfere with the measurement of soil organic ^{13}C unless removed or excluded from measurement. We report a simple and convenient HCl-fumigation method to remove inorganic C from soil. Soil samples are weighed in Ag-foil capsules, arranged on a microtiter plate, wetted with water to approximately field capacity, and placed in a desiccator containing a beaker with concentrated (12 M) HCl. The carbonates are released as CO_2 by the acid treatment in 6 to 8 h. The soil samples are then dried at 60°C prior to isotope determination. The advantages of the HCl-fumigation method to remove inorganic C from the soil are that: (i) no water soluble C will be lost from the soil; (ii) a large number of samples can be processed simultaneously; (iii) the removal of inorganic C is rapid and complete; and (iv) the method could also be used to determine both organic and inorganic C content in the soil. A potential disadvantage, however, is that the HCl fumigation changed the ^{15}N natural abundance of soil N.

THE $\delta^{13}\text{C}$ METHOD is increasingly being used to follow C dynamics in the soil (Balesdent and Mariotti, 1996; Collins et al., 1999). The difference between the ^{13}C content of the existing soil organic matter (SOM) and the new plant C may result from changes in the photosynthetic pathway of vegetation (C3 vs. C4) (Balesdent and Mariotti, 1996; Collins et al., 1999), or when ^{13}C depleted CO_2 is used to elevate the atmospheric CO_2 concentration such as in a free atmospheric CO_2 enrichment (FACE) experiment (Leavitt et al., 1994; Van Kessel et al., 2000a,b). The difference in the $\delta^{13}\text{C}$ values of the new C input and of the older SOM C is sufficiently large to follow the dynamics of both the new and the old C in the soil (Balesdent et al., 1988).

In addition to organic C, soil may also contain inorganic C in the form of carbonates. The C in primary or lithogenic carbonates has $\delta^{13}\text{C}$ values close to 0‰ Pee Dee Belemnite (PDB) (Boutton, 1991). Pedogenic or secondary carbonates show $\delta^{13}\text{C}$ values between -12 and $+2$ ‰ (PDB), depending on the photosynthetic

pathway of vegetation (C3 vs. C4). When a sample is combusted at high temperature (1000°C), all organic and inorganic C present in the soil is converted into CO_2 . To avoid the confounding influence of inorganic C during the determination of the isotopic signature of the organic C, all carbonates must be removed prior to isotopic analysis. Because carbonates may be enriched in ^{13}C by as much as 30‰ compared with organic C, partial removal of carbonates will have a large effect on the $\delta^{13}\text{C}$ signature of the sample. For example, if residual carbonate at 0‰ accounts for 1% of total soil C in a soil where organic C is -25 ‰, the inclusion of the CO_2 in the measurement would result in a 0.25‰ error.

A common method to remove carbonates from soil is treatment with dilute HCl or H_3PO_4 (Connin et al., 1997; Rochette and Flanagan, 1997; Collins et al., 1999; Van Kessel et al., 2000b). Although acid washing will remove all carbonates, the procedure is time consuming and could lead to losses of acid-soluble organic C. Thus, there is a risk that the soluble C may be isotopically different from the insoluble residue. However, Midwood and Boutton (1998) found that the $\delta^{13}\text{C}$ signature of SOM C was largely unaffected by the acid concentration (0.1–6 M HCl) or duration (1–8 d) of the acid treatment. To avoid losses of soluble organic C and possible changes in the $\delta^{13}\text{C}$ signature of SOM, the removal of carbonates can be carried out by HCl fumigation (Hedges and Stern, 1984).

Using continuous flow isotope ratio mass spectrometers, it has been common to determine both the ^{13}C and ^{15}N isotopes from a single combusted sample (Nadelhoffer and Fry, 1988; Van Kessel et al., 1994). However, the possibility that HCl fumigation changes the ^{15}N isotopic composition of the sample must be investigated if C and N isotope measurements are to be routinely applied.

The main objectives of this study were (i) to determine the effectiveness of HCl fumigation in removing carbonates from soil, (ii) to test the effects of HCl treatments on the residual SOM ^{13}C , and (iii) to determine the effects of the HCl treatment on $\delta^{15}\text{N}$ of soil N. The possible use of the HCl-fumigation method to determine inorganic and organic C contents was also explored.

Materials and Methods

To determine the rate and effectiveness of carbonate removal by HCl vapor, a calcareous soil with a high inorganic C content was selected (Table 1). The calcareous soil (Mollisol) was oven-dried and ball-milled for 24 h. Subsamples (30 mg) of soil were placed in open Ag-foil capsules (8 by 5 mm). Silver capsules are required because Sn capsules disintegrate when exposed to HCl vapor. The capsules were placed in the wells of a microtiter plate, sufficient water was added to each capsule (50 μL) to moisten the soil to approximately field

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Table 1. Physical and chemical characteristics of the soils used for the HCl fumigation and washing experiment.

Soil order and description	Common name	Horizon	pH	Total C		Total N		Sand	Silt	Clay	CEC
				mg g ⁻¹		g kg ⁻¹					
Inceptisol: loamy, mixed, superactive, thermic Lithic Haploxerepts	Auburn	A†	5.8	29.1	2.5	460	320	120	12.2‡		
Inceptisol: loamy, mixed, active, mesic shallow, Typic Dystroxerepts	Chawanakee	A†	5.8	24.8	1.3	180	90	630	6.4‡		
Inceptisol: loamy skeletal, mixed, superactive, mesic, Typic Haploxerepts	Pardaloe	A§	6.2	26.2	1.3	210	430	360	24.7		
Entisol: fine silty, siliceous, active, acid, thermic, Fluvaquentic Endoaquepts	Pophers	A¶	4.3	50.7	4.3	10	600	390	3.6		
Mollisol: clay-loamy, mesic, Argiudoll	Unknown	C	8.1	25.8	3.6	320	370	310	NA#		

† Data from Trott, 1981.

‡ Includes only Ca and Mg.

§ Data from Munn and Singer, 1981, p. 93.

¶ Data from Midwood and Boutton, 1998.

NA = not available.

capacity, and the microtiter plate was then placed in a vacuum desiccator (5 L). A beaker (150 mL) with 100 mL of concentrated (12 M) HCl was also placed inside the desiccator. The samples were exposed to HCl vapor for times increasing in 6-h increments to 30 h, then 12-h increments to 96 h. After each exposure time, four replicates were removed from the desiccator, dried at 60°C for 4 h, and the capsules were then closed.

The effects of HCl fumigation and HCl washing on total soil N and C content and ¹³C and ¹⁵N abundance were determined on four carbonate-free soils: Auburn (loamy, mixed, superactive, thermic Lithic Haploxerepts), Chawanakee (loamy, mixed, active, mesic, shallow Typic Dystroxerepts), Pardaloe (loamy-skeletal, mixed, superactive, mesic Typic Haploxerepts), and Pophers (fine-silty, siliceous, active, acid, thermic Fluvaquentic Endoaquepts) (Table 1). Subsamples of the soil were analyzed either untreated, exposed overnight to HCl vapor, or treated with 0.5 M HCl (10 mL g⁻¹ soil) for 24 h followed by two washings with water.

The procedure of Midwood and Boutton (1998) was followed for acid washing the soils. Briefly, 5 g of ball-milled soil was treated with 150 mL of 0.5 M HCl and the soil-acid mixture stirred three times over a 24-h period. Soils were then washed twice with distilled water. Each time the water was replaced after a 24-h period. Soil was dried at 60°C, ground by mortar and pestle, 30-mg samples were weighed into Ag foil capsules, and the capsules closed for isotopic analysis. Four replicates were used for all analyses.

Total N, total C, ^δ¹⁵N, and the ^δ¹³C for all samples were determined on a Europa 20-20 continuous flow isotope ratio mass spectrometer (PDZ Europa Ltd., Sandbach, UK) following combustion at 1000°C in a Europa ANCA-GSL CN analyzer (PDZ Europa Ltd., Sandbach, UK). The ^δ¹⁵N values are

expressed relative to atmospheric N₂. The ^δ¹³C values are expressed relative to Vienna-Pee Dee Belemnite (V-PDB). Controls (Ag capsules, HCl fumigation and water) were included. The amounts of C and N in the controls never exceeded 2 μg for N and 6 μg for C, and were too low to obtain a reliable isotopic composition.

One-way analysis of variance followed by the Student-Newman Keuls test (SAS Institute, 1989) was used to test for differences in total N, total C, ^δ¹⁵N, and ^δ¹³C between treatments.

Results and Discussion

A common practice for removing carbonates from soil is washing the samples with dilute acid followed by several washings with deionized water (Connin et al., 1997; Midwood and Boutton, 1998; Collins et al., 1999). Although, the ^δ¹³C of the bulk soil organic C may be unaffected by this treatment (Midwood and Boutton, 1998), it is conceivable that the composition of isotopically heterogeneous soils could be altered by this treatment through removal of dissolved organic C. Fumigating the soil with HCl may reduce or eliminate any organic C losses and minimize the potential for changes in ^δ¹³C.

Acid fumigation with HCl of the four noncalcareous soils did not alter their ¹³C compositions or their organic C and total N contents (Table 2). Treating the same soils with dilute HCl followed by washing led to significant declines in total soil C and N for all four soils. For three soils, the ^δ¹³C value also became more negative. As the decline in the ¹³C value for these three soils ranged

Table 2. Results of HCl fumigation and HCl washing on the elemental and isotopic N and C in soil (n = 4 replicates).

Soil name	Treatment	^δ ¹⁵ N	^δ ¹³ C	N		C	
				‰		mg g ⁻¹	
Auburn	Untreated	1.80 ± 0.01a†	-26.98 ± 0.02a	2.528 ± 0.000a	29.138 ± 0.004a		
	HCl fumigation	1.84 ± 0.05a	-26.93 ± 0.01a	2.455 ± 0.000a	28.960 ± 0.008a		
	HCl washed	1.97 ± 0.04b	-26.96 ± 0.02a	2.366 ± 0.001b	27.743 ± 0.017b		
Chawanakee	Untreated	0.78 ± 0.04a	-26.02 ± 0.01a	1.286 ± 0.002a	24.826 ± 0.026a		
	HCl fumigation	0.84 ± 0.03a	-26.04 ± 0.01a	1.300 ± 0.001a	25.124 ± 0.007a		
	HCl washed	0.81 ± 0.02a	-26.11 ± 0.01b	1.174 ± 0.002b	23.099 ± 0.027b		
Pardaloe	Untreated	2.70 ± 0.02a	-25.90 ± 0.02a	1.251 ± 0.000a	26.206 ± 0.008a		
	HCl fumigation	2.81 ± 0.03b	-25.92 ± 0.01a	1.167 ± 0.001a	26.147 ± 0.018a		
	HCl washed	2.84 ± 0.03b	-26.06 ± 0.02b	1.139 ± 0.001b	23.881 ± 0.017b		
Pophers	Untreated	2.58 ± 0.02a	-27.31 ± 0.02a	4.260 ± 0.002a	54.090 ± 0.028a		
	HCl fumigation	2.64 ± 0.01a	-27.28 ± 0.01a	4.175 ± 0.001a	53.158 ± 0.026a		
	HCl washed	3.38 ± 0.04b	-27.51 ± 0.01b	3.570 ± 0.004b	48.584 ± 0.045b		

† Means ± S.E. within a column and followed by the same letter are not significantly different at P < 0.05.

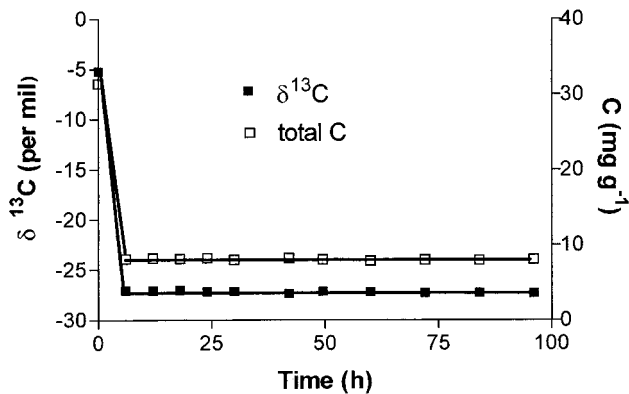


Fig. 1. Changes in soil C concentration and the ^{13}C content of soil C following the removal of carbonates by HCl fumigation. The open squares represent C values, the closed squares represent $\delta^{13}\text{C}$ values.

between 0.09 and 0.20‰, the removed C was enriched in ^{13}C compared with residual organic C. When coralline sediments that contained >80% by weight CaCO_3 were exposed to HCl fumigation, no contamination or loss of organic C and N occurred (Yamamuro and Kayanne, 1995). In contrast, when samples were acidified with 20 mL of 1 M HCl and washed with distilled water, 20% of the C and N was lost. Since those samples were not analyzed for isotopic composition, it remains unknown whether the ^{13}C and ^{15}N abundances were changed. Midwood and Boutton (1998) used one of the carbonate-free soils included in this study (Pophers). When they treated this soil with 1 M HCl for up to 8 d, followed by washing the soil with distilled water, organic C concentration declined significantly but the $\delta^{13}\text{C}$ value of the soil was unaffected.

Acid fumigation always increased the $\delta^{15}\text{N}$ value of the soil but for only one soil (Pardaloe) was the increase significant at $P < 0.05$ (Table 2). For this soil, there was also a decrease, albeit not a significant one, in total N content following HCl fumigation (Table 2). Apparently, the amount of N lost remained small but its $\delta^{15}\text{N}$ differed sufficiently to result in an increase in the $\delta^{15}\text{N}$ value of the bulk soil N following fumigation. Lohse et al. (2000) observed up to a 50% loss of N in continental margin sediments following the elimination of carbonates by acidification using diluted H_2SO_4 . Fumigation with HCl led to a small but consistent increases in the $\delta^{15}\text{N}$ value of soil N and losses of N can occur. Therefore, some caution is recommended in the use of ^{15}N measurements from HCl-fumigated soils. Treating soil with diluted HCl followed by washing significantly reduced the N concentration for all soils tested, and the $\delta^{15}\text{N}$ value increased significantly in three of the four soils (Table 2). Midwood and Boutton (1998) also observed significant losses in N concentration when the Pophers soil was treated with HCl. Clearly, when soils are washed with HCl, total N concentration can be reduced, and the $\delta^{15}\text{N}$ changed.

Exposure to concentrated HCl vapor for 6 h completely removed carbonates from a soil that contained high levels of carbonates (Fig. 1). The high $\delta^{13}\text{C}$ value (-5‰) of untreated soil reflected a strong presence of carbonates. The $\delta^{13}\text{C}$ value declined to -27‰ following

HCl fumigation for 6 h. No further decline in the $\delta^{13}\text{C}$ value occurred for the remainder of the fumigation period. At the same time, the total C content of the soil declined from a maximum of 32 mg g^{-1} to 8 mg g^{-1} after 6 h of HCl fumigation and remained constant thereafter (Fig. 1). However, Hedges and Stern (1984) found that HCl-vapor treatment failed to remove all carbonate from a soil with 50% carbonate, suggesting that HCl fumigation may not always be completely effective in removing carbonates from highly calcareous soils.

Water should be added to the dried soil prior to the HCl fumigation. In dry soil, the rate at which carbonates are converted into CO_2 is low and the treatment may fail to remove all carbonates from the soil (data not shown). In this study, the addition of $50 \mu\text{L}$ of distilled water to 30 mg of soil was sufficient to ensure complete removal of carbonates upon HCl fumigation.

The HCl fumigation technique is a convenient and easy method to remove inorganic C and could potentially also be used to determine the quantity of organic and inorganic C in a soil. One sample of the soil should be fumigated with HCl, another sample left untreated. Following total C analysis, the difference in total C content in the soil is attributed to carbonates. Total soil C content in the calcareous soil before HCl fumigation was 32 mg g^{-1} soil and decreased to 8 mg g^{-1} soil after HCl fumigation. Therefore, 24 mg g^{-1} soil or 75% of the total C content in the soil was in the form of inorganic C.

It should be pointed out that the HCl-fumigation method for determining inorganic C requires two separate analyses: total organic and inorganic C (untreated sample), and organic C (treated sample). As both analyses contribute experimental error, the calculated amount of inorganic C based on the difference between the C in the two analyses may be less accurate than a direct measurement. However, this point requires further research.

Finally, it should be mentioned that the HCl-fumigation method does reduce, by about 50%, the number of samples that can be combusted before the elemental analyzer combustion reactor is exhausted.

Conclusions

Fumigating soil with HCl vapor rather than washing the soil in dilute HCl is an effective method to remove carbonates prior to isotopic analysis. The method does not alter the $\delta^{13}\text{C}$ signature of organic C and no losses of organic C occurred. In a soil that contained 75% of total C as carbonates, all carbonates were removed within a 6-h period of fumigation. A large number of samples can be processed simultaneously. However, HCl fumigation may lead to a small increase in the $\delta^{15}\text{N}$ value of soil N.

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References

- Balesdent, J., and A. Mariotti. 1996. Measurement of soil organic matter turnover using ^{13}C natural abundance. p. 83–112. *In* T.W.

- Boutton and S. Yamasaki (ed.) Mass spectrometry of soils. Marcel Dekker, New York.
- Balesdent, J., G.H. Wagner, and A. Mariotti. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. *Soil Sci. Soc. Am. J.* 52:118–124.
- Boutton, T.W. 1991. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. p. 173–185. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, San Diego, CA.
- Collins, H.P., R.L. Blevins, L.G. Bundy, D.R. Christensen, W.A. Dick, D.R. Huggins, and E.A. Paul. 1999. Soil carbon dynamics in corn-based agroecosystems: Results from carbon-13 natural abundance. *Soil Sci. Soc. Am. J.* 63:584–591.
- Connin, S.L., R.A. Virginia, and C.P. Chamberlain. 1997. Carbon isotopes reveal soil organic matter dynamics following arid land shrub expansion. *Oecologia* 110:374–386.
- Hedges, J.L., and J.H. Stern. 1984. Carbon and nitrogen determination of carbonate-containing solids. *Limnol. Oceanogr.* 29:657–663.
- Leavitt, S.W., E.A. Paul, B.A. Kimball, G.R. Hendrey, J.R. Mauney, R. Rauschkolb, H. Rogers, K.F. Lewin, J. Nagy, P.J. Pinter, Jr., and H.B. Johnson. 1994. Carbon isotope dynamics of free-air CO₂-enriched cotton and soils. *Agric. For. Meteorol.* 70:87–101.
- Lohse, L., R.T. Kloosterhuis, H.C. de Stighter, W. Helder, W. van Raaphorst, and T.C.E. van Weering. 2000. Carbonate removal by acidification causes loss of nitrogenous compounds in continental margin sediments. *Mar. Chem.* 69:193–201.
- Midwood, A.J., and T.W. Boutton. 1998. Soil carbonate decomposition by acid has little effect on the δ¹³C or organic matter. *Soil Biol. Biochem.* 30:1301–1307.
- Munn, J.R., Jr., and M.J. Singer. 1981. Relative erodibility of California benchmark soils. Tech. Rep. USDA–SCS. Land, Air and Water Resources papers 10007, Univ. California-Davis.
- Nadelhoffer, K.J., and B. Fry. 1988. Controls on natural nitrogen-15 and carbon-13 abundance in forest soil organic matter. *Soil Sci. Soc. Am. J.* 52:1633–1640.
- Rochette, P., and L.B. Flanagan. 1997. Quantifying rhizosphere respiration in a corn crop under field conditions. *Soil Sci. Soc. Am. J.* 61:466–474.
- SAS Institute. 1989. SAS/STAT user's guide. Version 6. 4th ed. Vol. 2. SAS Inst., Cary, NC.
- Trott, K.E. 1981. The importance of selected soil physical and chemical properties for the prediction of soil erodibility of several western upland soils. Ph.D. diss. Dept. of Land, Air and Water Resources, Univ. of California, Davis, CA.
- Van Kessel, C., R.E. Farrell, and D.J. Pennock. 1994. Carbon-13 and nitrogen-15 natural abundance in crop residues and soil organic matter. *Soil Sci. Soc. Am. J.* 58:382–389.
- Van Kessel, C., W.R. Horwath, U. Hartwig, D. Harris, and A. Lüscher. 2000a. Net soil carbon input under ambient and elevated CO₂ concentrations: isotopic evidence after four years. *Global Change Biol.* 6:435–444.
- Van Kessel, C., J. Nitschelm, W.R. Horwath, D. Harris, F. Walley, A. Lüscher, and U. Hartwig. 2000b. Carbon-13 input and turnover in a pasture soil exposed to long-term elevated atmospheric CO₂. *Global Change Biol.* 6:123–135.
- Yamamuro, M., and H. Kayanne. 1995. Rapid direct determination of organic carbon and nitrogen in carbonate-bearing sediments with a Yanaco MT-5 CHN analyzer. *Limnol. Oceanogr.* 40:1001–1005.

DIVISION S-10—WETLAND SOILS

Heterotrophic Microbial Activity in Northern Everglades Wetland Soils

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ABSTRACT

Phosphorus loading to the northern Florida Everglades has been implicated in causing changes in vegetation, peat accretion rates, and other soil physical-chemical properties. Our study focused on determining the influence of P loading on aerobic and anaerobic heterotrophic microbial activities (measured as CO₂ and CH₄ production) in detritus and soil collected from the Water Conservation Area 2a (WCA-2a) of the Everglades. Heterotrophic microbial activities measured under both field and laboratory conditions were higher in areas impacted by P loading as compared to the unimpacted interior marsh. Microbial heterotrophic activities were higher in detritus and surface soils and decreased with depth. In field studies, CO₂ production rates in anaerobic soils were approximately 64% of those observed in aerobic soils. Additions of substrates containing C, N, and P generally enhanced heterotrophic microbial activity. In laboratory studies involving addition of various inorganic electron acceptors, increased microbial activities in the order of O₂⁻ > NO₃⁻ = SO₄²⁻ > HCO₃⁻ were observed. Microbial CO₂ production rates under denitrifying and sulfate reducing conditions ranged from 30–42% and 29–44%, respectively, of aerobic rates. Methane production rates were only up to 9% of aerobic CO₂ production rates. Both CO₂ and CH₄ production rates were significantly correlated with soil P parameters and microbial biomass. Enhanced heterotrophic microbial activities resulting from P loading has the potential to increase turnover of organic matter which may lead to increased supply of bioavailable nutrients to emergent macrophytes and periphyton and higher nutrient concentrations in the water column.

ORGANIC MATTER DEGRADATION plays an important role in nutrient cycling of wetlands. Rates of organic matter degradation and CO₂ production provide an indication of the microbial activity of soils, with primary factors influencing microbial activity being concentrations of utilizable substrates, electron acceptors, and nutrients such as N and P (Webster and Benfield, 1986; D'Angelo and Reddy, 1994; Reddy et al., 1999).

A major limiting factor of microbial growth in short term studies is the utilization of readily degradable compounds of the dissolved organic carbon (DOC) pool (Hoppe, 1983). Short term studies primarily determine microbial respiration on the basis of the DOC pool. In long term studies, utilizable portions of the DOC pool are depleted, and heterotrophic microbial activity measurements are often based on utilization of large organic compounds which must be acted upon by extracellular enzymes, resulting in lower microbial activity (Chrost, 1991).

Organic matter degradation in wetlands is often limited by the availability of electron acceptors rather than electron donors (Reddy and D'Angelo, 1994; Amador and Jones, 1995; McLatchey and Reddy, 1998). Oxygen is the most important electron acceptor in terrestrial systems, but is usually limited to the upper soil surface and the overlying water column in wetlands. A sequential reduction of electron acceptors with depth in soils generally proceeds in the order of O₂⁻ > NO₃⁻ > SO₄²⁻ > HCO₃⁻ on the basis of theoretical thermodynamic energy yields to microorganisms (Billen, 1982; Reddy and D'Angelo, 1994). Thus, degradation rates of DOC in

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